# Experimental shift in diet $\delta$13C: a potential tool for ecophysiological studies in marine bivalves

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# Experimental shift in diet $\delta^{13}C$: a potential tool for ecophysiological studies in marine bivalves

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**Abstract** - To test the potential of diet switching experiments in ecophysiological studies of marine invertebrate, stable carbon isotope ratios were measured at different seasons in the gonad, adductor muscle, digestive gland and gills of scallops (*Pecten maximus*) and oysters (*Crassostrea gigas*) held for 15 days on a constant diet of phytoplankton depleted in $^{13}C$. The aim of this study was to determine if differences in carbon incorporation could be detected among species, seasons and organs, and if so, whether it was consistent with their known energy-allocation patterns. After offering the new diet, isotope values of the different organs gradually shifted and significant differences among organs, seasons and species were found. A carbon incorporation index (CII) was calculated to compare the metabolic activity of each organ of the two species between day 0 and day 15. For both species, the digestive gland had the highest CII, the adductor muscle the lowest, while gonad and gills had intermediate values. The CII was generally much higher in *P. maximus* than in *C. gigas*, suggesting higher metabolic activity in this species. Seasonal differences in the CII were also observed for the two species and were interpreted as differences in metabolic activity in accordance with our energy allocation scenario. Therefore stable isotope diet switching experiments appear to be of great value for assessing metabolic orientation in bivalves.

Keywords: energy allocation, metabolism, carbon isotopes, *Pecten maximus*, *Crassostrea gigas*

# 1. Introduction

Knowledge of metabolic activity and energy allocation strategies for bivalves is of great interest, particularly for aquaculture. Indeed, metabolic pathways are directly or indirectly linked to processes important for survival or reproduction (Dalhoff 2004). Therefore, understanding the origin and fate of nutrients provides a mechanistic basis for successful rearing and reproduction of bivalves in controlled conditions for aquaculture purposes. For example, the same broodstock conditioning schedule repeated at different seasons of the year generally gives rise to many different results in terms of fecundity and hatching success for many mollusc species (e.g. Utting and Millican 1997; Robert and Gérard 1999), thus revealing large seasonal trends in physiology of these organisms and making knowledge of their metabolic orientation of primary importance.

General studies have provided data from single-point measurements of respiration, assimilation, excretion and organ weights (Vahl 1981a,b; Bayne et al. 1983; MacDonald and Thompson 1985a,b, 1986, 1987). However, those measures give only instantaneous data, and the hypothetical energy allocation scenarios produced from these types of measurements offer only approximations of net production and never depict the real carbon and energy fluxes from the environment to the animal, nor among organs within the animal. Therefore, development of new tools in bivalve ecophysiology appears of primary importance.

Stable isotope techniques (e.g., C, N, H, S), usually applied in ecological and population biology studies (reviews by Peterson and Fry 1987; Michener and Schell 1994), have already been used with success in experimental studies to investigate energy allocation patterns (O'Brien et al., 2000; Gauthier et al., 2003; Voigt et al., 2003). These techniques are based on the assumption that the isotopic composition of an organism is linked to that of its diet. Generally, experimental protocols involve diet switching from one isotopically distinct

72 diet to another. The main principle is that the speed at which the isotopic value of an organ
73 changes after a diet switch is a function of the metabolic activity of the organ, including both
74 turnover and growth. For example, such an approach has been successfully applied in
75 determining energy allocation to reproduction in moths (O'Brien et al., 2000), and tissue
76 turnover in fishes (Herzka and Holt 2000; Bosley et al. 2002, Suzuki et al., 2005).

77 Previous work on the scallop *Pecten maximus* (Linné 1758) in the field showed that
78 tissue isotopic composition can be influenced by metabolic activity of the organism (Lorrain
79 et al. 2002). We therefore expected that carbon incorporation rates would be affected by
80 changes in bivalve energy demand and allocation. These carbon incorporation rates could then
81 be followed in several tissues of individuals reared at different periods of the year by
82 measuring $\delta^{13}C$ after a diet switch. To test the potential of stable isotope experiments to
83 effectively track carbon incorporation in bivalve species, an isotope diet switching experiment
84 was carried out under controlled conditions. This work was conducted at four different
85 periods of the year and on two different species, *P. maximus* and the oyster *Crassostrea gigas*
86 (Thunberg 1793). These two species, intensively studied for aquaculture purposes, are known
87 to show distinct seasonal behaviour, scallops having a highly regulated annual oscillation of
88 reserve storage and utilisation (Saout 2000), whereas oysters tend to have a more
89 opportunistic strategy of energy allocation (Enriquez-Diaz 2004). We therefore expect that the
90 seasonal patterns of carbon incorporation would be more pronounced in *P. maximus* than in
91 *C. gigas.* We chose several target tissues because of their different physiological functions
92 and the likelihood of differences in carbon incorporation: adductor muscle, gonad, digestive
93 gland and gills for both species; and the remaining tissues (i.e. labial palps, mantle and
94 perigonadic tissues) for oysters.
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## 2. Materials and methods

### *2.1. Collection of bivalves*

Both scallops and oysters were collected in western Brittany (France). Two-year-old scallops (86 ± 5mm, N = 48) were dredged in the Bay of Brest (Roscanvel, 48°20'N, 4°30'W), whereas oysters of approximately the same age (102 ± 10mm, N = 48) were hand collected at low tide in the Aber Benoît (Landéda, 48°34'N, 4°37'W). Sampling was carried out in March, May and September 2002, and January 2003 corresponding to the four different experimental periods (Table 1).

### *2.2. Diet switching experiments*

Experiments were carried out at the IFREMER Shellfish laboratory at Argenton (Finistère, France) in 2002 and 2003, utilizing cultured unicellular algae with low $\delta^{13}C$ (caused by bubbling $CO_2$ from a commercial cylinder into the culture medium) as a food source.

Four experiments were conducted, each one during a different hypothetical temporal window of energy allocation for scallops and oysters (i.e. in March, June, September and January; Table 1). Experiments lasted 15 days to minimize the possible effect of laboratory acclimation and to be sure that they would reflect natural metabolism and windows of energy allocation. After collection, bivalves were placed in 700 litre tanks with 1 µm filtered running seawater for two days during which time they were not fed to empty the digestive tract. Afterwards, three individuals were randomly chosen and sacrificed (to represent day 0). The

122 remaining animals were then offered a mixed diet of four unicellular algal species depleted in
123 $^{13}C$ (25% *Chaetoceros calcitrans*, 25% *Skeletonema costatum,* 25% *Isochrysis galbana*
124 named *T-iso*, 25% *Tetraselmis chui)*. During all four experiments, this diet was supplied *ad*
125 *libitum* using a continuous dripping device with a daily ration equal to 8 % dry weight
126 algae/dry weight flesh of animal. Algal concentrations were verified each day. A mixed diet
127 was preferred to a single species diet as tissue production and normal rearing are reduced and
128 perturbed with single species diets (Utting and Millican 1997; Robert and Gérard 1999). Each
129 experiment was conducted at the ambient water temperature (Table 1).
130 At each sampling date (in general days 0, 2, 6 and 15), three individuals were taken for
131 testing. Stomachs of scallops were first rinsed with a few millilitres of 0.2 µm filtered
132 seawater injected via the mouth, to completely purge digestive tracts (see Lorrain et al. 2002
133 for more details). Gonad, adductor muscle and digestive gland were then dissected from each
134 individual. As not considered as organs of predominant role in bivalve energy strategies, gills
135 of the three individuals were pooled in one sample. For oysters, the remaining tissues (mantle,
136 labial palps and perigonadic tissues), generally considered as a storage tissue for this species,
137 were also collected. As these remaining tissues were not sampled in scallop, results from the
138 remaining oyster tissues will be regarded only as a first attempt to confirm the potential
139 storage role of these tissues. All samples were frozen at -20°C until analysis.
140 Dietary isotopic composition was monitored by measuring the stable isotope signature
141 of algae samples (Table 1). These algae were sampled by filtering 15 ml of the mixed algae
142 through a precombusted Whatman GF/F filter (nominal porosity = 0.7 µm) at different
143 periods of the experiments. The filters were then stored dried in clean glass vials after 12
144 hours at 60°C until analysis.
145
146 *2.3. Isotopic analyses*

After freeze drying, bivalve tissue samples were ground to a homogeneous powder and 1 mg samples were folded into 6×4 mm tin cups for continuous flow - isotope ratio mass spectrometer (IRMS) analysis. Analysis was performed using a Europea Scientific ANCA-NT 20-20 Stable Isotope Analyser with ANCA-NT Solid/Liquid Preparation Module (PDZ Europa Ltd., Crewz, UK, Scottish Crop Research Institute, Dundee, Scotland). The analytical precision (SD, N = 5) was 0.2 ‰ for C, estimated from standards analysed along with the samples. Triplicate analyses performed on some samples confirmed that analytical reproducibility was very good (0.2 ‰ maximum variation). All isotopic data are given in the conventional delta notation in units of parts per thousand (‰) relative to the Vienna Pee Dee Belemnite (VPDB) standard as follows:

$$\delta^{13}C_{sample} = (R_{sample} / R_{standard} - 1) * 1000 \text{ where } R = {}^{13}C/{}^{12}C$$

Filtered algal samples were exposed to HCl vapour for 4 h at room temperature to remove carbonates (Lorrain et al. 2003). The filters were then folded, placed into 9×5 tin cups and kept in closed vials until analysis. The samples were analysed for C content and isotope ratios by N. Naulet at the University of Nantes (LAIEM, UMR CNRS 6006, France) using a Carlo Erba NA 2100 elemental analyser coupled to a Finnigan Delta S IRMS. Analytical reproducibility performed on ten replicate filters was better than 0.2 ‰ (see Lorrain et al. 2003).

*2.4. Carbon incorporation index*

To evaluate the differences in carbon incorporation among seasons, organs and species, we calculated a carbon incorporation index (CII). To express the actual net carbon change relative to the maximal expected change, such an index should be calculated as:

$$CII = [\delta^{13}C_{d15} - \delta^{13}C_{d0}] / [\delta^{13}C_{diet15} - \delta^{13}C_{diet0}] * 100$$

where $\delta^{13}C_{d0}$ = the tissue $\delta^{13}C$ value at the beginning of the experiment, $\delta^{13}C_{d15}$ = the tissue $\delta^{13}C$ value at day 15; $\delta^{13}C_{diet0}$ = the diet $\delta^{13}C$ value before the beginning of the experiment and $\delta^{13}C_{diet15}$ = the $\delta^{13}C$ value of the diet during the experiment.

In fact, the food carbon isotopic ratio before the start of the experiment ($\delta^{13}C_{diet0}$) remains unknown. We therefore calculated the CII by replacing $\delta^{13}C_{diet0}$ by $\delta^{13}C_{d0}$ *i.e.* the tissue $\delta^{13}C$ value at the beginning of the experiment:

$$CII = [(\delta^{13}C_{d15} - \delta^{13}C_{d0}) / (\delta^{13}C_{diet} - \delta^{13}C_{d0})] * 100$$

When the tissue $\delta^{13}C$ values were not available for day 15, a linear extrapolation based on the slope obtained from the two preceding values was used. For example, in September, muscle $\delta^{13}C_{d15} = \delta^{13}C_{d14} + [(\delta^{13}C_{d14} - \delta^{13}C_{d6}) / 8)]$. $\delta^{13}C_{diet}$ represents the average $\delta^{13}C$ value of the diet during the 15 days of the experiment (Table 1).

This index integrates growth and turn-over processes and therefore gives an idea of the quantity of metabolites allocated to a specific organ. This CII does not take into account eventual differential fractionation factors between organs, or some isotopic routing processes, as is discussed later.

### *2.5. Data analysis*

Differences in carbon isotope composition between d0 and d15 were tested by performing a non-parametric Kruskal-Wallis test for each organ (except for the remaining tissues in March and the gills for which the analyses were conducted on pooled samples) and each experiment. The same method was applied for CII comparison. When significant differences were detected, results were classified using the Mann-Whitney non parametric procedure. Differences were considered significant at $\alpha = 0.05$.

## 3. Results

The decrease of the tissue $\delta^{13}C$ over time in both species and for nearly all organs, indicates successful incorporation of dietary carbon from phytoplankton (Fig. 1 and 2). Significant carbon isotope change between d0 and d15 was observed for digestive glands, adductor muscles and gonads of scallops in all seasons (Fig. 1, $p<0.05$). For oysters, significant change was also observed in all experiments ($p<0.05$) except for adductor muscle during the June experiment and gonad during the September experiment (Fig. 2, $p>0.05$). Within this general decreasing trend, some differences between species, seasons, and organs were apparent. For example, in March, scallop and oyster digestive gland tissues showed the most rapid decrease of $\delta^{13}C$ over time, with an average of 11.3 and 5.8 ‰ change in 12 days, whereas muscle showed only 1.6 and 0.9 ‰ for scallops and oysters, respectively (Fig. 1 and 2). These differences can be expressed by the carbon incorporation index (CII, see Methods). The CII clearly reveals a strong difference between scallops and oysters (Fig. 3). Scallops always had a larger CII than oysters ($p < 0.01$), irrespective of organs and seasons, except for the digestive gland and muscle in January, when oysters had a higher CII than scallops.

Muscle had always the lowest CII, except for oysters in June and September (Figure 3, Table 3) where muscle did not differ significantly from remaining tissues and gonad, respectively. Conversely, digestive gland always had the highest CII values, except for scallops in March and September when gonad and digestive gland did not differ significantly. Gonads, gills and remaining tissues showed intermediate values between muscle and digestive gland.

Seasonal differences do appear in CII, but differ between species. In scallops, a general trend toward increasing values from March to January experiments was observed. In

oysters, seasonal variations are less pronounced although experiments conducted during winter months produced significantly higher CII values compared to other months (Table 3).

## 4. Discussion

As expected, diet-switching experiments led to changes over time in tissue carbon isotopic composition, confirming the potential of stable isotope studies to trace carbon within organisms. Indeed, the carbon incorporation index (CII, see Methods) shows significant differences among organs, species and seasons. We should stipulate that this CII gives combined information of tissue growth and turnover (see Gannes et al., 1997), which we could not separate in this study. Furthermore, as already mentioned in materials and methods, it would have been better to calculate the CII using the food $\delta^{13}C$ value before the diet switching, instead of using the tissue $\delta^{13}C$ value at the beginning of the experiment. Further experiments should try to evaluate this, even if it is difficult when using bivalves in their natural environment. However, these global data can still provide valuable information about metabolite allocation to an organ, which can be very useful for ecophysiological studies.

Furthermore, even though the general idea that $\delta^{13}C$ variations are directly linked to organ activity and growth and can help us to study bivalve metabolism, some other processes may account for the observed differences and reduce the power of this study. According to the literature, two main processes acting on $\delta^{13}C$ variations should be considered: isotopic routing and differential isotopic fractionation (Gannes et al., 1997).

Isotopic routing is a process by which some biochemical components from the diet, with specific isotopic signatures, are preferentially allocated to certain organs. Most of the time, lipid accumulation is proposed to explain some decreases in $\delta^{13}C$ as lipids are strongly

247 depleted in $^{13}C$ (Tieszen et al., 1983). To determine if such a process can fundamentally affect
248 interpretation of our data, we are able to simulate the effect of a complete isotopic routing,
249 i.e., only dietary lipids incorporated in one organ. To perform such a simulation, the isotopic
250 composition of the lipid and non-lipid fraction of the diet has to be known. Data from the
251 literature allows us to estimate that $\delta^{13}C$ of the lipid fraction of a tissue is around 6 to 7 ‰
252 lower than of its non-lipid fraction (McConnaughey and McRoy, 1979; Kling et al., 1992;
253 Bearshop et al., 2002). By a mass balance calculation, we estimated the $\delta^{13}C$ value of the lipid
254 fraction of the diet in June (phytoplankton with a maximum 20 % of lipids: Whyte, 1987;
255 Brown, 1991) to be about -50.8 ‰, whereas the non-lipid fraction is about -42.4 ‰. We
256 hypothesize that the digestive gland, which is known to be an organ rich in lipids (30 %,
257 Saout et al., 1999), exclusively incorporates the lipid fraction of the diet (estimated $\delta^{13}C$ =
258 -50.8 ‰) and conversely that the muscle (a fat-free organ) incorporates only the non lipid
259 fraction ($\delta^{13}C$ = -42.4 ‰). Then using these new values in place of the measured dietary $\delta^{13}C$,
260 we can re-estimate the CII. For the muscles of scallops in June, the CII values would shift
261 from 3.9 to 4.1 %, and for the digestive gland from 45.4 to 34.7 %. In January, recalculated
262 muscle values would not change significantly whereas digestive gland values would shift
263 from 17.5 to 14.3 %. Therefore, this estimation of the maximum isotopic routing would
264 explain only up to 30 % of the observed variations and would not change our conclusions.
265 Differing fractionation factors between organs could also impact the CII results, but
266 data for differential fractionation between organs of the same organism are scarce (see
267 Dalerum and Angerbjörn, 2005) and even nonexistent for marine bivalves. However, the
268 results of Lorrain et al. (2002) suggest that these differing fractionation factors would not
269 exceed 4 ‰ in scallops (between muscle and digestive gland). Therefore, including differing
270 fractionation factors (from 1 to 4 ‰) to the $\delta^{13}C_{diet}$ value in the CII calculations would have a
271 very small impact on CII value differences observed between organs.

272 The CII differed among organs in the following order: digestive gland > gonad >
273 adductor muscle. Furthermore, seasonal variations are stronger in the digestive gland and in
274 the gonad than in the muscle, suggesting that the digestive gland and the gonad integrate
275 shorter time variations as compared to the muscle. On an annual average, the gills presented
276 intermediate CII values, close to those of the gonad (23 and 9 % for scallops and oysters
277 respectively) and were constant throughout the year. The "remaining tissues" CII presented an
278 annual mean value close to 7 % but with large seasonal variations, suggesting that these
279 tissues might have a storage role, in contrast to the gills.

280 The most striking result of our diet switching experiments is that strong differences in
281 the CII were observed among seasons. Food, provided ad libitum in all the four experiments,
282 cannot account for these differences. Temperature differed between experiments, but was not
283 the cause of these variations as both the maximum and the minimum scallop CII were
284 observed at the lowest temperature (Fig. 3), in March and January, respectively. To a lesser
285 extent, the same inference can be drawn for oysters. From this, it can be concluded that
286 carbon allocation is not driven by immediate thermal conditions, as is suggested for many
287 bivalves (McDonald and Thomson, 1985b, 1986). Our results constitute the first data set
288 illustrating this pattern for bivalves obtained using stable isotope diet switching experiments.

289 Variations between the two species CII probably reflect their different energy
290 allocation strategies. From several works on the same scallop population of the Bay of Brest
291 (Paulet et al. 1988, 1997; Saout et al. 1999; Saout 2000), a hypothetical schedule of metabolic
292 activities can be drawn (Fig. 4). Scallops in the Bay of Brest are characterized by a strategy of
293 storage and postponed use of energy. Basically, energy stored as glycogen in the adductor
294 muscle and principally as lipids in the digestive gland during spring and summer, is used to
295 sustain reproductive effort and maintenance during winter. In spring and summer, somatic and
296 reproductive production is directly fuelled from the available food. Schematically, in terms of

297 energetic allocation priority, the year for an adult scallop can be subdivided in three main
298 parts (Fig. 4): i) from November to April, metabolic translocation from somatic to
299 reproductive tissues occurs, ii) from April to May, a transitory period, is characterized by the
300 simultaneous production of somatic and reproductive tissues mainly from food, and iii) from
301 June to October, major energetic fluxes originate from the food and are used for reserve
302 building.

303 In contrast to the scallop, the Japanese oyster, *C. gigas*, from the coast of Brittany,
304 exhibits an annual cycle by which food can directly sustain growth and reproduction for most
305 of the year (Chavez-Villalba et al. 2001; Chavez-Villalba et al. 2002a, b; Enriquez-Diaz
306 2004). Spring and summer are periods of major gonadal production, whereas somatic growth
307 occurs during this period according to a more opportunistic manner depending on food
308 availability (Fig. 4). Reproductive activity is sustained either by direct uptake or via
309 metabolites stored in the reserve tissues ("remaining tissues": mantle, labial palps and
310 perigonadal tissues). In oysters, seasonally based biological changes are acknowledged to be
311 less rigid than for scallops, with individuals inhabiting a large range of environmental
312 conditions (Enriquez-Diaz, 2004).

313 For scallops in the present study, the maximum carbon incorporation in reserve
314 tissues, i.e. muscle and digestive gland, is observed in March and June. Intense incorporation
315 into the gonad is limited to March and to a lesser extent to June and September. These results
316 show that energy allocation to reproduction is observed in March and that reproduction can
317 still occur in the second temporal window of energy allocation (June to September) in
318 accordance with the annual schedules shown in figure 4. Therefore, contrary to previous
319 hypotheses, scallops seem able to assimilate external food as early as March, and not only
320 after April; this probably reveals the opening of a “receptive window” to food availability
321 somewhere between the end of January and the beginning of March. The very low CII values

observed for somatic tissues in September is contradictory to the prediction of the energy allocation model (Fig. 4), and probably reveals an overestimation of the storage process in autumn even though target organs (muscle and digestive gland) are near their maximum filling. Finally, carbon incorporation is at its minimum in January for all tissues, underscoring the fact that gonadic activity observed in winter for this species (Paulet et al. 1997) is directly dependant on the use of storage tissues.

In oysters, CII values are generally lower than in scallops and seasonal differences were less pronounced. In this species, carbon incorporation in the gonad revealed by isotope results was maximal in March and June, in agreement with the energy allocation model (Fig.4) and verifying that gonadal tissue develops during this period, at least partially from food uptake. In September, the gonad CII remains close to zero, corresponding precisely to the resting stage documented for this species in Brittany (e.g. Lango-Reynoso et al. 1999; Li et al. 2000; Fig. 4). Seasonal variations of the CII of the digestive gland appear less pronounced than in scallops, in accordance with its relatively minor role as a storage organ in oysters compared to scallops. However, the high CII observed for the digestive gland relative to the other organs in all seasons suggests that this organ would have a more important storage role in oysters than previously described. The “remaining tissues” exhibited a high CII value in March, probably due to their predominant role as a reserve compartment. This heterogeneous tissue composed of the mantle, the labial palps and the perigonadal envelope will be the object of more extensive studies in the future. For both species, the gills represent a site of active incorporation during all seasons, which warrants further studies.

Our results from carbon isotope tracing agree with previous knowledge on energy allocation for the studied bivalves. Clearly scallops appear as a species with more rigid and contrasted temporal allocation windows than oysters, as evidenced by the greater seasonality in the CII. This apparent highly regulated functioning might be compared with the existence

347 of a putative annual rhythmic physiological oscillation, driven or not by photoperiod, as
348 proposed by Paulet and Boucher (1991) for this species. One must also consider that scallops
349 have very distinct periods of energy allocation, using food or tissue reserves, whereas oysters
350 use both food and tissue reserves simultaneously.

351 Another striking result of these experiments was the generally lower magnitude of the
352 CII for oysters as compared to scallops. The carbon incorporation, discussed in this study, is a
353 double source process: i) the renewal of existing tissues (tissue turnover), and ii) the
354 production of new tissues (growth). This must be considered when discussing differences in
355 the CII between both species. Although growth was not measured during the course of the
356 experiment, tissue growth data for oysters in Aber Benoît (Fleury et al., 2001) and for scallops
357 in the Bay of Brest (Lorrain et al., 2004) reveals that at the same age, a scallop produces
358 annually at least two times more soft tissues than an oyster. Therefore, a part of the observed
359 difference in CII values could be due to differences in tissues growth between the two species.
360 In future studies, the development of methods adapted to precisely assess tissue production at
361 the individual level would be of primary importance. Secondly, the markedly lower CII
362 observed for oysters could also reveal a lower metabolism, inducing a lower tissue turnover,
363 for this species compared to scallops. Such a difference seems consistent with some other life
364 history traits of these species, such as i) the potential mobility in scallops contrasted with the
365 sedentary life of the oysters, and ii) the larger pallial cavity, and the greater valve movements,
366 in scallops compared to oysters of the same size, probably allowing higher pumping rates in
367 scallops (Møhlenberg and Riisgård, 1979; see also discussion in Bricelj and Shumway, 1991).

368 Finally, for dietary studies, in which stable isotopes are a key tool, this kind of
369 experiment could be continued over longer time periods to assess turnover and fractionation
370 factors between food and different tissues. Indeed, the bivalves sampled at the end of our
371 experiments had not yet reached equilibrium with their new diet. Furthermore, in this study,

372 the elucidation of great differences in carbon incorporation kinetics between organs confirms
373 the potential of multi-organ analyses to study spatial or temporal variations in diet $\delta^{13}C$
374 (Tieszen et al., 1983; Hobson and Clark, 1992a, b; Hobson et al. 1996). Indeed, to study
375 trophic dynamics at different time scales, the digestive gland and gonads are more appropriate
376 than the muscle to detect short-term food source variations, as the muscle only gives an
377 average value over a long period.
378

379 ## 5. Conclusion

380

381 Diet switching experiments, conducted under the same diet regime but at different
382 periods of the year, have revealed differences in carbon incorporation among organs, seasons,
383 and species. These results are consistent with previous knowledge on energy allocation
384 strategies for *P. maximus* and *C. gigas*. This study represents an important first step in
385 establishing the potential of stable isotope diet switching experiments for carbon tracing in
386 bivalves. In this regard, information from this type of experiment would offer valuable
387 insights into bivalve ecophysiology and energy allocation patterns. The next stage will be the
388 coupled study of isotope tracking with a whole carbon budget of the two species, including
389 consumption, respiration, production (organ by organ) and faecal excretion estimations.
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393

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529 Table captions

530

531 Table 1. Details of the experimental protocol of the four experiments (March, June,
532 September and January): exact dates, water temperature, isotopic composition of the diet
533 (means ± 1 S.D, N for number of measurements) and specific composition of the unicellular
534 algae species that compose the diet given to scallops and oysters.

535

536 Table 2. Results of the multiple range test for differences in CII among organs for the same
537 experiment (organ effects) and among experiments for the same organ (seasonal effects) for
538 scallops (A) and oysters (B). ns: non significant, ** : significant at the 95% level, nd: not
539 determined. G: Gonad; DG; Digestive Gland; M: Muscle; R: Remaining tissues.

540

| | March 2002 | June 2002 | September 2002 | January 2003 |
|---|---|---|---|---|
| Dates | 8 to 23 March | 30 May to 14 June | 9 to 24 September | 10 to 25 January |
| Temperature | 10°C | 14°C | 17°C | 10°C |
| Diet $\delta^{13}C$ (‰) | -42.7<br>N = 1 | -43.9 ± 0.5<br>N = 2 | -50.3 ± 3.1<br>N = 3 | -52.8 ± 2.7<br>N = 4 |
| Unicellular algal species | *Isochrysis galbana, Skeletonema costatum, Tetraselmis chui, Chaetoceros calcitrans* | | | |

541

## A. *Pecten maximus*

Organ effects

| | March | | June | | September | | January | |
|---|---|---|---|---|---|---|---|---|
| | G | DG | G | DG | G | DG | G | DG |
| M | ** | ** | ** | ** | ** | ** | ** | ** |
| G | | ns | | ** | | ns | | ** |

Seasonal effects

| | Muscle | | | | Gonad | | | | Digestive Gland | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | March | June | Sept | | March | June | Sept | | March | June | Sept |
| June | ** | | | June | ** | | | June | ** | | |
| Sept | ** | ns | | Sept | ** | ** | | Sept | ** | ** | |
| Jan | ** | ** | ** | Jan | ** | ns | ** | Jan | ** | ** | ns |

## B. *Crassostrea gigas*

Organ effects

| | March | | | June | | | September | | | January | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | G | DG | R | G | DG | R | G | DG | R | G | DG | R |
| M | ** | ** | nd | ** | ** | ns | ns | ** | ** | ** | ** | ** |
| G | | ** | nd | | ** | ** | | ** | ** | | ** | ns |
| DG | | | nd | | | ** | | | ** | | | ** |

Seasonal effects

| | Muscle | | | Gonad | | | Digestive Gland | | | Remaining Tissues | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | March | June | Sept | March | June | Sept | March | June | Sept | March | June | Sept |
| June | ns | | | ns | | | ns | | | nd | | |
| Sept | ns | ns | | ** | ** | | ** | ns | | nd | ** | |
| Jan | ns | ns | ns | ** | ** | ** | ** | ** | ** | nd | ** | ns |

557 Figure captions

558

559 Figure 1. *Pecten maximus*. Stable carbon isotope values ($\delta^{13}C$, in ‰) of scallops as a function
560 of time since the diet switch for the four different experiments (March, June, September and
561 January), each sub-figure corresponding to one of the four organs (adductor muscle, gills,
562 gonad and digestive gland). Values are means ± 1 standard deviation (N = 3) except for gills
563 where the value corresponds to a pool of the three individuals.

564

565 Figure 2. *Crassostrea gigas*. Stable carbon isotope values ($\delta^{13}C$, in ‰) of oysters as a
566 function of time since diet switch for the four different experiments (March, June, September
567 and January), each sub-figure corresponding to one of the four organs (adductor muscle, gills,
568 gonad, digestive gland) and the remaining tissues (mantle, labial palps and perigonadic
569 tissues). Values are means ± 1 standard deviation (N = 3) except for gills where the value
570 corresponds to a pool of the three individuals.

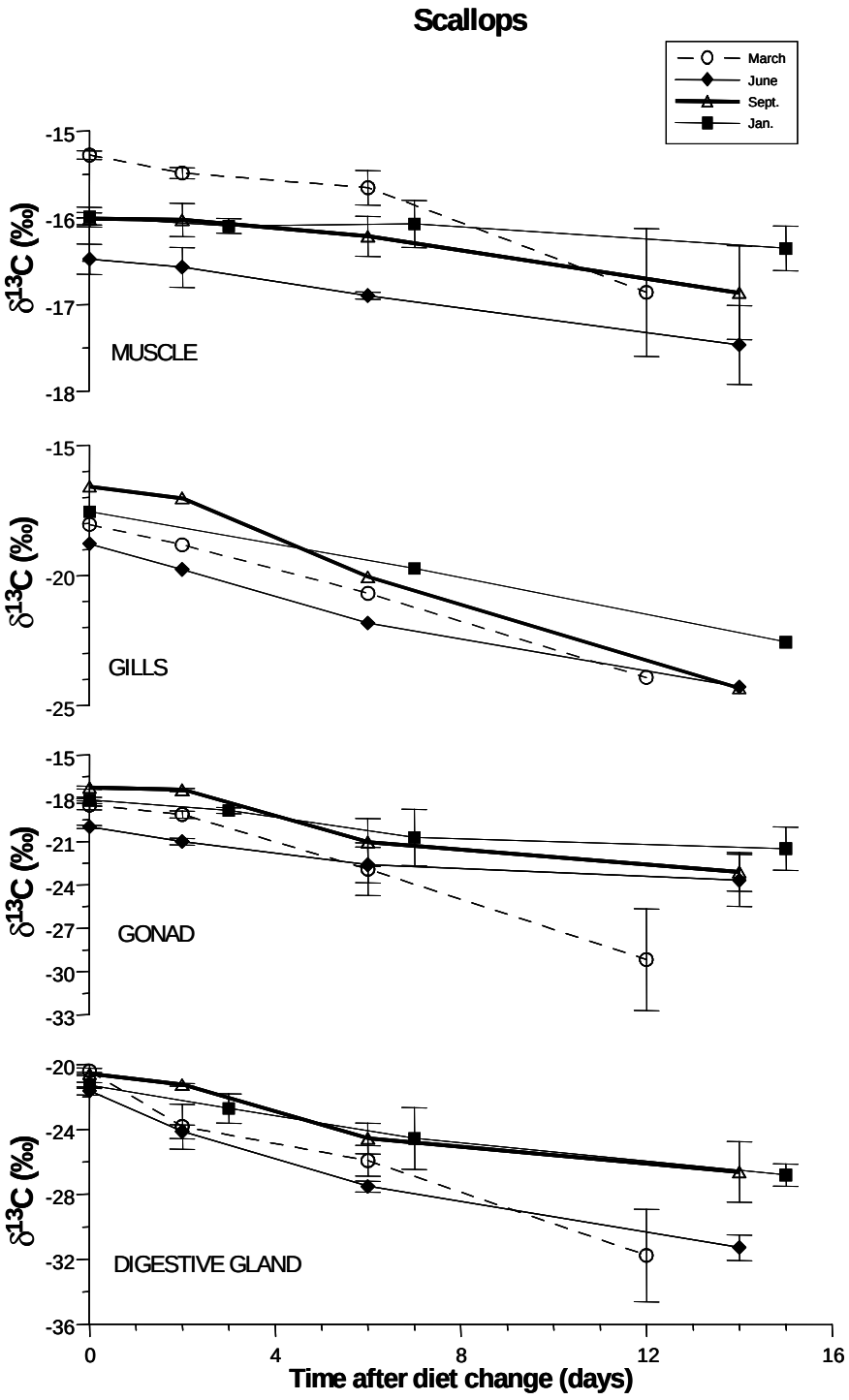
571

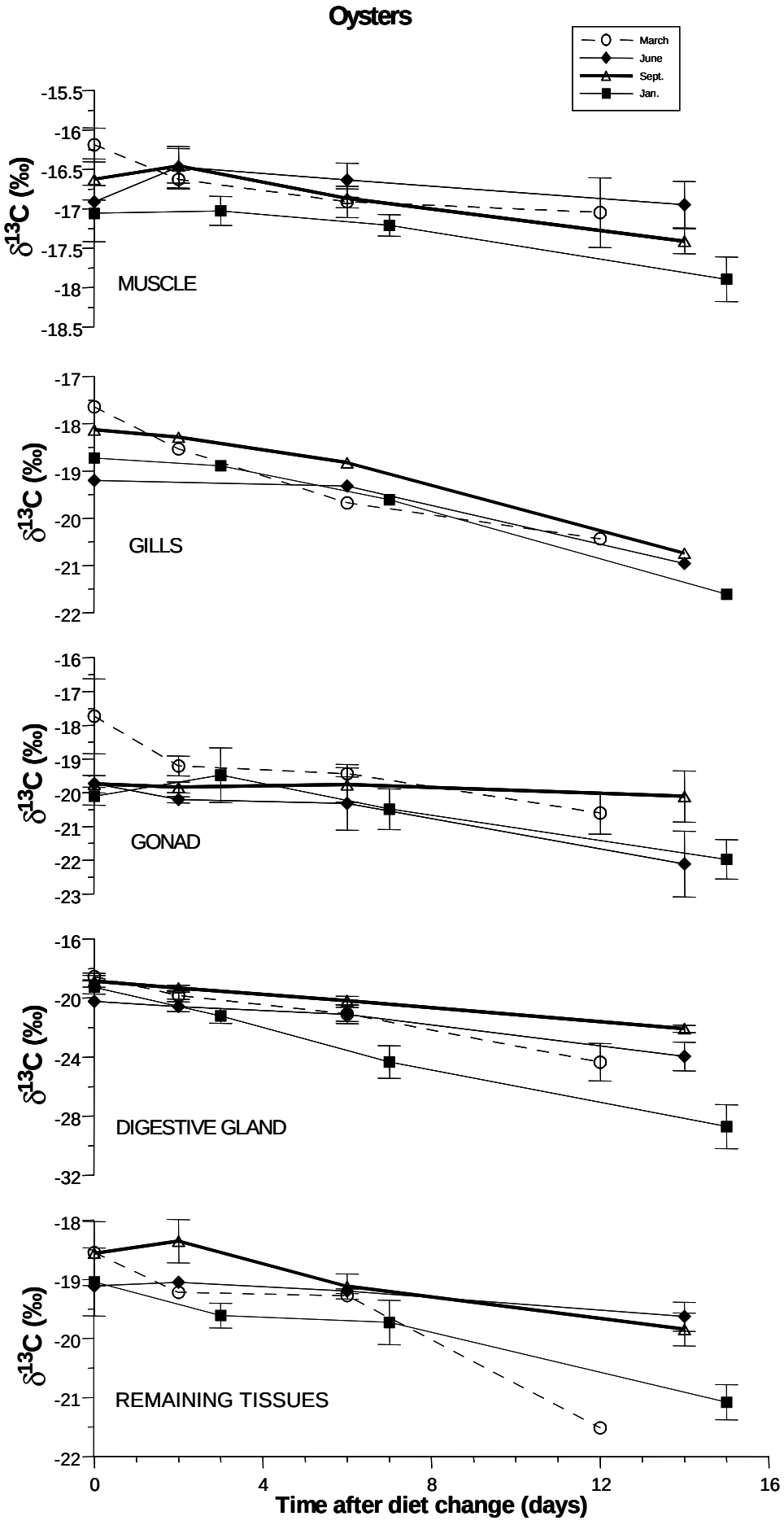
572 Figure 3. Carbon Incorporation Index (CII, in %) in the different organs (adductor muscle,
573 gonad, digestive gland, gills and remaining tissues) of *P. maximus* (black bars) and *C. gigas*
574 (grey bars) for the different experiments (March, June, September and January). Temperature
575 during experiments is also indicated. See materials and methods for calculations of CII.
576 Standard deviations are indicated when available.

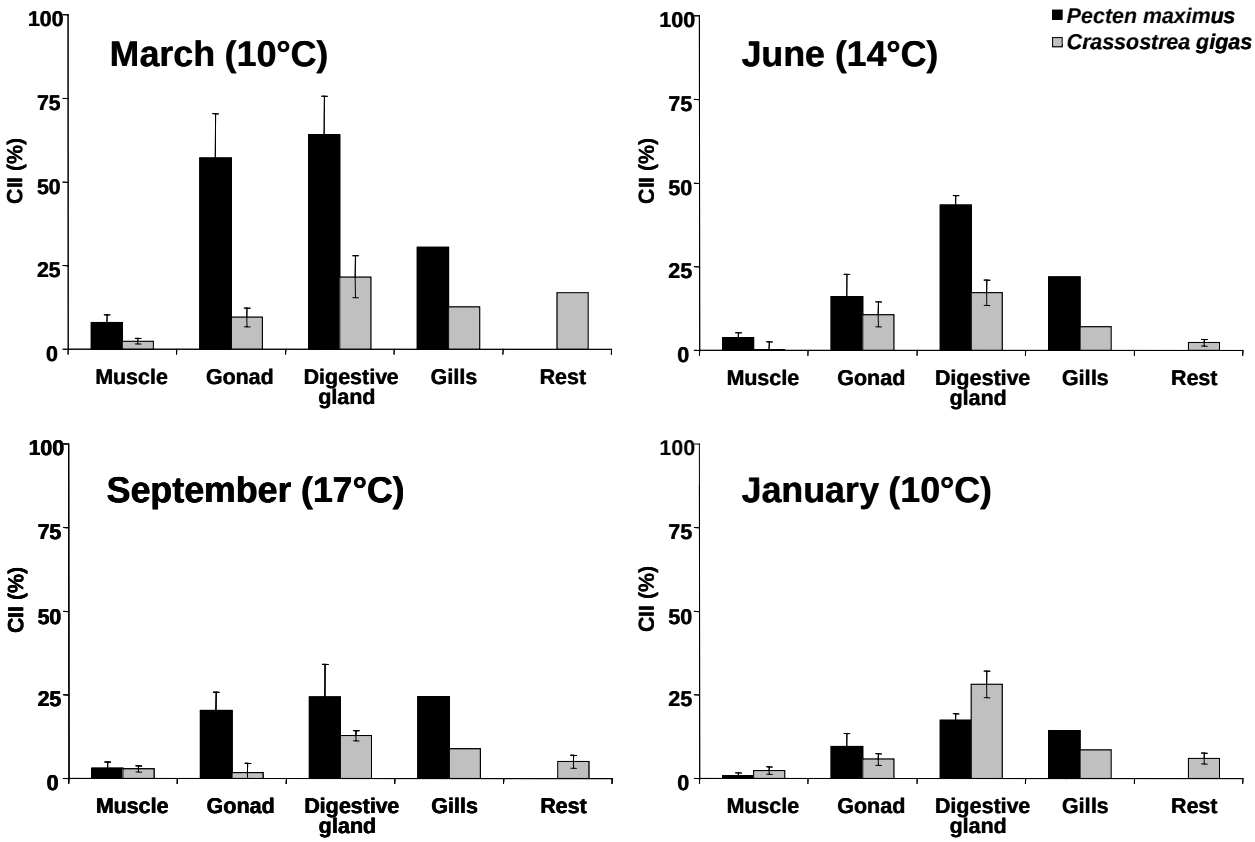
577

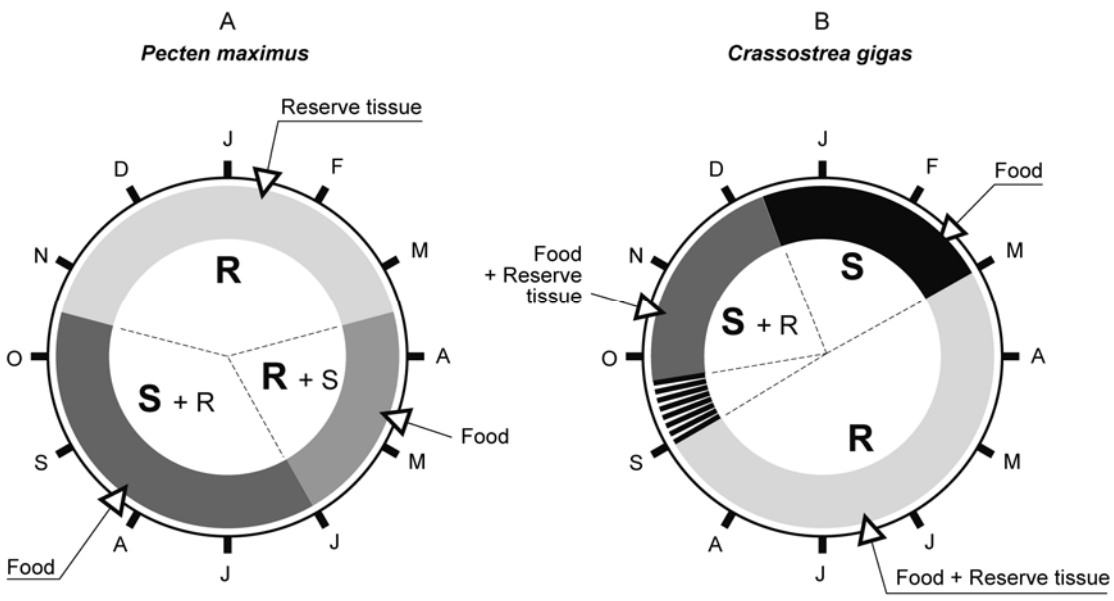
578 Figure 4. Hypothetical annual model of energy allocation for the two bivalves species
579 developed from previous studies (see discussion) A) *Pecten maximus* and B) *Crassostrea*
580 *gigas*. Arrows illustrate energy origin (food or reserve tissue) during the three different
581 periods for each species. R signifies that energy is primarily being allocated to reproduction

582 and S to somatic growth; small caps indicate secondary processes that can still occur if energy
583 is in excess. Hachure section represents a resting stage for oysters.


Scallops
March
June
Sept.
Jan.
MUSCLE
GILLS
GONAD
DIGESTIVE GLAND
δ13C (‰)
Time after diet change (days)


Oysters
March
June
Sept.
Jan.
MUSCLE
GILLS
GONAD
DIGESTIVE GLAND
REMAINING TISSUES
δ13C (‰)
Time after diet change (days)


March (10°C)
June (14°C)
September (17°C)
January (10°C)
CII (%)
100
75
50
25
0
Muscle
Gonad
Digestive gland
Gills
Rest
Pecten maximus
Crassostrea gigas


A
Pecten maximus
Reserve tissue
J
F
M
A
M
J
J
A
S
O
N
D
R
S + R
R + S
Food
Food
B
Crassostrea gigas
Food
Food + Reserve tissue
S
S + R
R
Food + Reserve tissue

584